Metal Mixtures and Potential Environmental Health Risks in an Urban Superfund Site

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Abstract

Butte, Montana, a town of approximately 35,000 residents, is a part of the largest superfund site in the United States. One of the unique aspects of Butte's history is that surface mining and smelting activities occurred, and continue today, in close proximity to Butte's historically dense urban population. A preliminary study using geographic and demographically chosen volunteers found several elements (copper, manganese, and molybdenum) to be elevated in the Butte population, with low levels of selenium and zinc compared with non-exposed controls. Biological sampling (nasal swabs) also indicated perturbation in miRNAs associated with oncogenesis (mir-126, 486, and 424), the NF-κB pathway (miR-449c-5p and miR-445a), and cell cycle progression (mir-424-5p, 449b-5p, and 451a). We propose to expand the preliminary study to include a larger and epidemiologically representative study group. We will also use the environmental data to direct tissue culture studies in order to establish that model for further detailed analysis of the biological effects of exposure to a mixed-metal profile. These preliminary data illustrate the need for a larger number of study participants and data collection of samples from individuals living downwind of an active open-pit copper mine to determine current environmental health risks.

ICP-MS Hair Analysis

Table 1. Average parts per million concentrations of 36 elements quantified from hair samples that were acid digested and analyzed by ICP-MS. Elements with an asterisk (*) were found to be statistically different between groups by a One-Way ANOVA test.

Selection Criteria and Methods

Butte volunteers were chosen based on the following criteria: be at least 18 years of age, have lived in Butte, MT for a minimum of 10 consecutive years, and be a nonsmoker. Bozeman volunteers were chosen based on similar criteria, barring persons having lived in Butte for any period of time.

- A total of 26 volunteers were selected for the study, 17 from Butte (9 females and 8 males) and 9 from Bozeman (5 females and 4 males).
- A minimum of 0.15g of hair was collected from each volunteer and placed in an envelope. The hair samples were acid digested and then analyzed by ICP-MS for a suite of 36 elements.
- Nasal samples were collected using a sterile brush and were immediately placed in a lysis buffer on dry ice until total RNA could be extracted.
- All volunteers filled out a lifestyle survey regarding exercise and food choices, as well as personal and family medical history.

Total RNA was isolated and purified. The resulting total RNA was sent to LC Sciences in Houston, TX, where all known human miRNA sequences were identified and quantified for each sample.

Of the 26 samples collected, only 20 were sent to LC Sciences. For unknown reasons, 6 samples suffered RNA degradation and were omitted from the study. Therefore, 12 samples from Butte (6 female and 6 male) and 8 control samples from Bozeman (4 female and 4 male) were analyzed. Statistical analysis was performed and expressed as a paired student t-test.

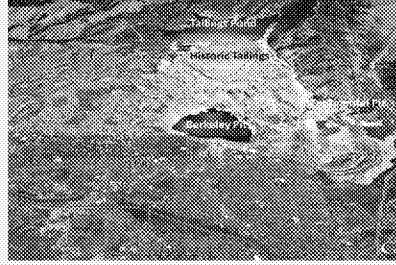All 26 hair samples were digested and analyzed by ICP-MS. One-Way ANOVA statistics were performed on the results.

Figure 1. Google Earth image of Butte, MT and the large surface mining activities within city limits. Images in red are overlays of one example of known areas with high levels of waste in place from historical mining practices.
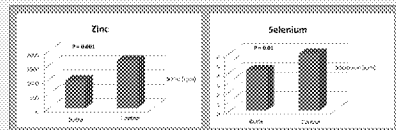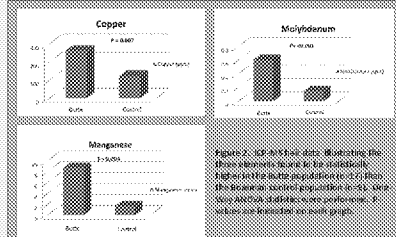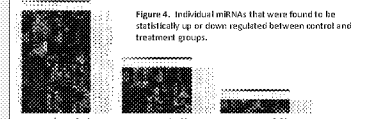

Figure 2. ICP-MS hair data illustrating the four elements found to be statistically higher in the Butte population (p < 0.05). One-Way ANOVA statistics were performed on each graph.

Figure 3. ICP-MS hair data illustrating the two elements found to be statistically lower in the Butte population (p < 0.05). One-Way ANOVA statistics were performed. P-values are indicated on each graph.

Table 2. Human miRNA Data. Total RNA was sent to LC Sciences and miRNA microarray analysis was performed for all known mature miRNAs. MicroRNA values shown in red are statistically up-regulated and those shown in blue are statistically down-regulated in the Butte population (treatment). Student t-test was used to generate statistics.

Figure 4. Individual miRNAs that were found to be statistically up or down regulated between control and treatment groups.

Conclusions

This preliminary study analyzed metal accumulations in the hair and miRNA expression patterns of individuals living downwind of an active copper and molybdenum open pit mine. In addition, this area has over 100 years of mining and smelting activity and there are known areas with high levels of waste in place. Volunteers from Bozeman, MT, a city without historical or current mining, were recruited to submit control samples. Seventeen volunteers from Butte, MT (9 females and 8 males) and nine volunteers from Bozeman, MT (5 females and 4 males) submitted hair samples for ICP-MS analysis. Copper, molybdenum, and magnesium were elevated in the Butte population while zinc and selenium were found to be statistically low. Twelve samples from Butte (6 females and 6 males) and eight samples from Bozeman (4 females and 4 males) were submitted to LC Sciences for microRNA microarray analysis. Each sample was analyzed for all known mature human miRNAs. MicroRNA data is inconclusive, but several miRNAs associated with oncogenes as well as inflammation, the NF-κB pathway, and cell cycle progression were found to be significantly changed between populations. This preliminary study illustrates the need for additional testing to fully understand the potential health risks to individuals living in this area.

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